VOLUME 19 NUMBER 8

NUTRITION REVIEWS

August 1961

Nutrition and Genes in the Domestic Fowl, by F. B. Hutt 225

Clinical Nutrition

Food Additives 227
Vitamin B_6-Dependency State in Infants 229
Electron Microscopic Studies in Whipple's Disease 230
Total Body Water in Malnourished Infants 232
Results of Treatment in Phenylketonuria 234
Protein Losing Enteropathy 236

Animal Nutrition

Radio Toxicity of Iodine^{131} in Dairy Cattle 237

Experimental Nutrition

Bound Niacin 240
Potassium Deficiency and the Kidney 242
Long-Term Feeding of Fats and Atherosclerosis in Chicks 244
Dietary Components and Accumulation of Radionuclides in the Body 245
Accelerated Growth of Rats 247
Regulation of Cholesterol Metabolism with Thyroxine Analogues ... 250
Lowering of Serum Cholesterol by an Adephenine Derivative 251
Radionuclides, Calcium, and Potassium in Milk 253

Notes

Blood Saccharoids 254
Environmental Temperatures and Urinary Metabolites of Noradrenaline 255
Recent Books 256

Published Monthly at Mt. Royal and Guilford Aves., Baltimore, Md.

for

THE NUTRITION FOUNDATION, INC.

99 PARK AVE. NEW YORK 16, N. Y.

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FREDRICK J. STARE, PH.D., M.D., Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, One Shattuck Street, Boston, Mass.

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Changes of address should be sent to The Nutrition Foundation.
Second-class postage paid at Baltimore, Md.

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NUTRITION REVIEWS

VOL. 19 AUGUST 1961 No. 8

NUTRITION AND GENES IN THE DOMESTIC FOWL

Over half a century ago, Christiaan Eijkman, the Dutch physician in charge of a prison hospital in Java, noticed that fowls fed on garbage from the hospital showed some of the same symptoms as did his patients suffering with beriberi. This led eventually to the discovery that the element needed to protect both hens and patients from that disease was in the germ of the grain. We have learned quite a lot about vitamin B_1, or thiamine, since those early days.

Following the discovery in Rumania that White Leghorns are able to withstand diets deficient in thiamine much longer than are Rhode Island Reds, chicks of these two breeds were tested and found to show the same difference. Whether started on thiamine-deficient diets at hatching or at later ages, the White Leghorns grew better and lived longer than chicks of heavy breeds in four separate trials. It was discovered later that White Leghorns put more thiamine into their eggs than do hens of heavy breeds like Plymouth Rocks and Rhode Island Reds fed the same diet. N. S. Scrimshaw *et al.* found thiamine to be 66 per cent higher in the yolk of Leghorn eggs. With another method of analysis and better sampling of the breeds, Howes and Hutt found 43 per cent more thiamine in Leghorn eggs than in those of three heavy breeds.

While hens and families differ significantly in thiamine content of their eggs, any one hen puts a fairly constant amount into the egg. The correlation between thiamine content of different eggs from the same hen is very high even in eggs laid a month apart.

Together all these facts show that the White Leghorn utilizes thiamine more efficiently than do heavier breeds, but as the tests with chicks showed, this difference is not related to body size.

White Leghorns can also cope better than heavy breeds with diets deficient in vitamins D or E. Within one breed, individual hens differ so much in requirement of vitamin D that, as N. Olsson found, some can keep laying throughout a Scandinavian winter without any supplement of vitamin D, while others cannot.

When vitamin E is deficient, usually as a result of its being oxidized in stored feed, some of the chickens fed the deficient diet develop encephalomalacia. Affected birds suffer from ataxia, and capillary hemorrhages are found in the cerebellum. The usual preventive treatment is to supply some antioxidant in the feed. Here again White Leghorns have an advantage. On diets conducive to the development of encephalomalacia, the proportion of that breed afflicted is significantly lower than in representatives of heavy breeds. In pedigreed birds, differences among sire families in incidence of encephalomalacia are so great as to suggest that, when the level of that disease is not over 20 per cent, the fundamental trouble is more likely to be genetic susceptibility than any deficiency of the diet.

Genetic variations in the utilization of riboflavin are diverse and intriguing. One investigator has reported finding significantly more riboflavin in eggs of White Leghorns than in eggs of New Hampshires. Within White Leghorns the feasibility has been demonstrated of differentiating by selection two different strains, one better able than the other to thrive on diets low in riboflavin. Apparently polygenes (multiple factors) were involved in this case, and they provided ample material for

selection in both directions. In other words, the difference between the two strains in requirement of riboflavin was a quantitative character, corresponding to quantitative differences in rate of growth, size, viability, and the like.

Apart from such genetic variation, there is a special complication whereby diets containing riboflavin ample for most incubating chick embryos are inadequate for those that form entirely black plumage. Like so many other differences in nutritional requirements, this was discovered more or less by accident when hatcherymen reported that some of their black chicks hatched with bare backs (*i.e.* no down), while those not solid black were free from that defect. Investigations revealed that up to 5 per cent of the black embryos die during the last week or so of incubation and show the typical clubbed down that is characteristic of a deficiency of riboflavin. Some chicks hatch with bare backs, and even those that appear normal have unusually short down. When the eggs containing such black embryos are given supplementary riboflavin in the air cell, their hatchability is markedly increased. A good problem for some biochemist is: what is there about the formation of melanin in large amounts that causes embryos trying to form it to die with typical symptoms of riboflavin deficiency?

The most intriguing genetic abnormality in utilization of a nutrient is the remarkable defect studied by A. J. G. Maw. He found that some White Leghorns are completely unable to transfer riboflavin from the blood to the egg, as do normal birds, and that embryos trying to develop in these deficient eggs died at ten to 14 days of incubation. When riboflavin was injected into the air cell, development was normal and the chicks hatched. Maw was able to show that the inability to put riboflavin into the egg is a simple recessive, autosomal trait. In fact, it is the only variation in the utilization of nutrients by the fowl known thus far to depend upon a single gene in the homozygous state. The latest word on this remarkable case (R. V. Boucher, E. G. Buss, and Maw, *Poultry Sci.* **38,** *1190-2 (1959)*) is that the genetically abnormal hens have the same amount of riboflavin in the blood as do ordinary hens that are not laying. However, unlike ordinary hens, they cannot raise the blood level of riboflavin at the onset of laying. It remains at about .008 mcg./gm., while in layers the riboflavin level rises to .434 mcg./gm. The corresponding figures for egg yolk are 0.41 mcg./gm. in the abnormal hens, compared with 4.30 for those that function normally. The defect is not completely recessive, for heterozygotes show levels of riboflavin in the blood and in the eggs that are intermediate between the two extremes just given.

Breeds of the fowl differ in requirement of manganese, a deficiency of which causes genetically susceptible chickens to develop perosis, or slipped tendon. Fifty p.p.m. of manganese may be necessary to protect some growing chickens of the heavy breeds, whereas only 35 p.p.m. will do the same for even the worst-stressed Leghorns. One single generation of mass selection will greatly increase the proportion of a flock susceptible to perosis. Breeds differ also in the utilization of calcium, and several investigators have found White Leghorns to produce eggshells that are thicker or stronger than those of heavy breeds.

Two examples of breed differences in the requirement and utilization of amino acids have been reported. On diets deficient in arginine, D. M. Hegsted *et al.* (*J. Biol. Chem.* **140,** *191 (1941)*) found that growth of White Leghorn chicks lags far behind that of heavy breeds, but when that amino acid is supplied, and especially if it is given along with glycine, the White Leghorns respond markedly. The rapidly feathering White Leghorns may have a special need of arginine for the formation of feathers. M. W. McDonald (*Austral. J. Agric. Res.*

8, *587* (*1957*)) has found that when diets deficient in DL-methionine have 0.1 per cent of that amino acid added, the response in White Leghorns is five times as great as in Australorps. The latter have the sex-linked type of slow feathering.

The genetic differences considered in this brief review, and others not yet recognized, may account for discrepancies between results of experiments in different laboratories. More studies are needed with pedigreed birds, so that familial differences in requirements of specific nutrients can be recognized. Meanwhile, enough variation is known in the fowl to suggest that in other species, including our own, similar genetic differences in nutritional requirements may be much greater among races and individuals than has been recognized. Perhaps even Jack Sprat and his wife had a sound genetic basis for their antithetical dietary preferences.

White Leghorns are in most respects better able than the heavy breeds to withstand nutritional deficiencies and are also more resistant to extreme heat and to two kinds of Salmonella infection. The reason for their biological superiority is still a mystery. Tests run thus far indicate that it is not associated with dominant white plumage, and hence is more likely a characteristic of all Leghorns regardless of color. It is possible that the Leghorns may be a product of centuries of natural selection under conditions of severe stress, but it is difficult to see how the ancestors of the modern heavy breeds could have fared any better. The elucidation of this mystery should provide interesting problems for biologists of various disciplines, including both geneticists and nutritionists.

Studies for which references are not cited above were reviewed by Hutt (*Genetic Resistance to Disease in Domestic Animals.* Cornell University Press, Ithaca (1958)) and pertinent references are given in that book.

F. B. HUTT, PH.D., D.Sc.
Professor of Animal Genetics
Department of Poultry Husbandry
N. Y. State College of Agriculture
Cornell University
Ithaca, New York

FOOD ADDITIVES

Food additives, properly used, will result in cheaper, more abundant, and higher quality foods available to a larger number of people.

Few topics have aroused more heated arguments than the question of adding substances to food. Industrial representatives point out that the quality, attractiveness, and nutritive value may be enhanced and, at the same time, the price and perishability reduced. On the other side, claims have been made that all manner of chemical substances are being added to our food without regard to the consequence.

Food additives may be classified under various categories. Some are added to retard spoilage, enhance flavor, or improve color. Others may alter the consistency of a product by emulsifying or thickening it. Some retard drying while others retain crispness. A few are added as nutritional supplements, such as thiamine to bread, iodine to salt, fluoride to water or vitamins A and D to milk. Another group of substances gains entry into food unintentionally. This group includes insecticides, hormones, antibiotics, and disinfectants. Other accidental additives have been traced to the containers or wrappers of food, which may allow adhesives, dyes or plasticizers to become mixed with the food.

Recently three articles have appeared

which explain the position taken by industry. In the first of these L. A. Coleman (*Arch. Envir. Health* **2,** *209* (*1961*)) discussed the confusion which has arisen from recent Congressional hearings. The author emphasized that industry desires to comply fully with all regulations, but requests that decisions regarding the safety of additives be made by experts in the Food and Drug Administration and not by members of Congress.

A second article by K. E. Mulford (*Arch. Envir. Health* **2,** *212* (*1961*)) reviewed the legislation pertinent to this topic, which includes the Pesticide Residue Amendment of 1954, the Food Additives Amendment of 1958, and the Color Additive Amendment of 1960. The problem of pesticide residue has been well controlled in recent years. The Food Additives Amendment relates to such direct additives as flavorings and vitamins and to the indirect additives such as adhesives mentioned above. However, the Color Additives Law is more sweeping and relates to any dye, pigment or substance capable of imparting color to a food, drug, cosmetic or the human body. The author objected to the "poisonous doctrine," an interpretation that a substance must be safe at any level to be admissible. He pointed out that many compounds, either natural or synthetic, have a level of toxicity which must not be exceeded.

For example, polyoxyethylene stearate, which is used widely as a bread preservative, is nontoxic when fed at the 20 per cent level to rats. This amount is vastly greater than any conceivable level of human consumption (*Nutrition Reviews* **13,** *220* (*1955*)), yet some doubt has been expressed regarding its safety. The author pointed out that many substances are allowed by the F. D. A. under the rule "generally recognized as safe" (G.R.A.S.). He concluded that among the needs of the F.D.A. and of industry are some rules which would allow for development of short-term tests to insure adequate safety, thus allowing short pilot trials to evaluate consumer acceptance, followed by long-term feeding studies to insure safety of prolonged use by the public. He particularly resented the F.D.A. ruling that is known as the "cancer clause," which states that "no one can tell how much of a carcinogen might produce cancer" and hence no detectable quantities can be allowed. He cited the example of selenium which as a trace element is a dietary essential, in larger quantities is a toxic substance, and in certain situations is a carcinogen.

In a third article, D. B. Hand (*Arch. Envir. Health* **2,** *219* (*1961*)) discussed the safe use of food additives. He suggested continued permission to add the G.R.A.S. substances, but felt that the greatest problems related to colors and flavors. He suggested that criteria for safety include: consideration of (1) natural occurrence, (2) ease with which a substance is metabolized or detoxified, (3) the chemical reactivity of the compound, (4) the level of use or consumption, and (5) the length of time the product has been in use without apparent deleterious effects. Tests for carcinogenicity are more difficult. He suggested that the F.D.A. define carcinogens, define analytical methods for their detection, and exercise judgement.

These problems have been discussed before. W. C. Hueper (*A.M.A. Arch. Path.* **62,** *218* (*1956*)) urged strict limitation of the number of food additives and stressed the need for required adequate labeling. He also urged that an active research program be started in order to evaluate each substance fully. P. L. Day (*Nutrition Reviews* **18,** *1* (*1960*)) discussed the inadequacies of governmental facilities and finances in view of the huge task assigned to the Food and Drug Administration.

Perhaps the most pertinent comment was that both industry and the governmental food agencies must continue to strive as they have in the past, to deserve public confidence by demonstrating competence and wisdom.

VITAMIN B_6-DEPENDENCY STATE IN INFANTS

A well-studied case of a rare, and as yet unidentified, metabolic abnormality resulting in convulsions.

It was only a decade ago that the importance of vitamin B_6 in the nutrition of infants was clearly established. Deficiency of this vitamin was shown to result in hyperirritability and convulsions, and in anemia. These effects were promptly alleviated when adequate amounts of vitamin B_6 were provided.

Shortly after the early descriptions of vitamin B_6 deficiency, a case was reported in which the need for vitamin B_6 appeared to be much greater than normal (A. D. Hunt, Jr. *et al.*, *Pediatrics* **13,** *140 (1954)*). This infant, who suffered convulsions, responded favorably to large doses of the vitamin, and his ailment was designated as "pyridoxine dependency."

Now, Charles R. Scriver (*Pediatrics* **26,** *62 (1960)*) presents a well-studied case of this new and quite rare syndrome, including a number of biochemical investigations.

A female infant began to have generalized convulsions, hyperirritability, and hyperacusis on the seventh day of life. Examination was otherwise normal. The pregnancy had been uneventful and the delivery normal. It is of interest that the mother had been given oral vitamin supplements throughout pregnancy, including 9 to 12 mg. per day of pyridoxine. Laboratory investigations revealed normal values for serum calcium and glucose, normal roentgenograms of the skull, and a sterile blood culture. The cerebrospinal fluid protein was moderately elevated (100 mg. per 100 ml.), although an examination several days later revealed a normal value. The electroencephalogram revealed an abnormal pattern.

The usual sedatives were only partially successful in controlling the seizures and hyperirritability. However, on three separate occasions dramatic relief of symptoms followed the administration of pyridoxine in doses of 10 to 100 mg. given either orally or intramuscularly. Moreover, the infant grew and developed normally and remained free of symptoms on a regimen of daily pyridoxine administration. Withdrawal of the medication was attempted on a number of occasions; each time symptoms of hyperirritability and convulsions returned within four or five days. In addition, electroencephalographic abnormalities appeared; these abnormalities disappeared upon resumption of pyridoxine.

Through trial and error it was determined that a daily dose of 8.5 mg. pyridoxine was insufficient, and that the infant thrived on a dose of 11 mg. per day. At the time of the report the infant, now 20 months of age, was in good health, and free of symptoms.

It would appear that the criteria for the diagnosis of vitamin B_6-dependency have been established in this patient. The author also had the opportunity of observing a sibling of this patient some four years previously. The sibling had similar symptoms of convulsions and irritability which failed to respond to a number of anticonvulsive drugs. Death occurred at age four months. Autopsy revealed pneumonia, and congestion and edema of the leptomeninges. There were astrocytic hyperplasia in the basal ganglia and cerebral cortex, and some nerve cell loss in the thalamus, but no findings indicative of a specific etiology for the intractable convulsive state. Pyridoxine therapy was not used for this patient. The mother had not received pyridoxine supplements during pregnancy. Scriver is of the opinion that this infant also suffered from vitamin B_6-dependency. Convulsions have

not occurred in an older sibling, nor in other members of the family.

A number of biochemical studies were carried out in the surviving infant. These included urinary excretion of vitamin B_6 and 4-pyridoxic acid; serum transaminase levels; response to tryptophan loading, including xanthurenic acid excretion; chromatographic study of amino acid excretion; and study of urinary excretion of inorganic sulphate and oxalic acid. Also of interest was the observation that clinical response to pyridoxamine was comparable to that of pyridoxine hydrochloride.

It was found that 33 to 58 per cent of an administered dose of pyridoxine, a normal value, could be recovered in the urine. However, only 30 to 50 per cent of the amount recovered was in the form of 4-pyridoxic acid, which is about half that found in normal infants. When no supplements of pyridoxine were given, and the diet estimated to provide 0.5 to 1.0 mg. daily, the total excretion of vitamin B_6 was 0.4 to 0.7 mg. daily, which is about ten times the normal excretion value. The possibility is thus raised of excessive renal losses of the unmetabolized vitamin. On the other hand, an excessive demand for vitamin B_6 by the brain could result in a smaller excretion of the oxidative product 4-pyridoxic acid.

Tryptophan loading in patients suffering from vitamin B_6 deficiency results in the appearance of xanthurenic acid in the urine. Such was not the case in the patient under study. It would thus appear that the mechanism by which interference with pyridoxine metabolism causes convulsions differs from that prevailing in the deficiency state.

No abnormalities in serum glutamic-oxalacetic or glutamic-pyruvic transaminase were found in this patient. Neither lymphopenia nor anemia were present, although it must be stated here that these abnormalities might not be expected to occur within just a few days of pyridoxine withdrawal.

Quantitative analyses of urinary amino acid excretion were made during periods of pyridoxine withdrawal and therapy. Two-dimensional paper chromatography revealed no abnormalities. However, some differences were found on ion-exchange column chromatography. A three- to four-fold diminution upon pyridoxine withdrawal was found for aspartic and glutamic acids, valine, isoleucine, leucine, phenylalanine, lysine, and arginine. These are among those amino acids which are reabsorbed to a high degree in the renal tubule. The high degree of variability in amino acid excretion in normal subjects, however, renders these changes of doubtful significance.

Some suppression of urinary oxalate excretion, but no change in inorganic sulphate, occurred during pyridoxine loading in this patient.

This report constitutes a rather thorough and comprehensive study of a single patient with vitamin B_6-dependency. The precise nature of the defect is not yet apparent. Excessive renal loss of the vitamin does not account for all of the findings in this patient. The author speculates that the condition describes another, as yet unidentified, "inborn error of metabolism."

ELECTRON MICROSCOPIC STUDIES IN WHIPPLE'S DISEASE

A peroral jejunal biopsy showed macrophages containing characteristic material with a complex and varied ultrastructure of membranes, granules, and vesicles. Discrete virus-like particles were adjacent to the macrophages.

Knowledge of various malabsorption syndromes has been considerably advanced by the introduction in recent years of instruments for the peroral biopsy of the intestinal epithelium (*Nutrition Reviews* **17**, *5* (*1959*)). This procedure, by making

available fresh material unaffected by autolysis, has stimulated study of the intestinal changes in various conditions associated with malabsorption.

One of these, Whipple's disease, has attracted increasing attention in the past ten years. First described by G. H. Whipple in 1907, the condition is characterized by steatorrhea with polyarthritis, progressive weight loss, and, usually, death. There are heavy deposits of fat and fatty acids in intestine and mesenteric lymph nodes, with dilatation of lacteals and mesenteric lymphatics. Masses of large macrophages stuffed with finely vacuolated as well as granular material are prominent in the lesions. Other serous membranes' are involved in addition to those of the joints, though this is often not clinically apparent.

It was 16 years before another case was reported, and 13 more years elapsed before the next report was acceptable to P. Farnan, who has published an excellent review of the clinical aspects of this condition (*Quart. J. Med.* **28,** *163* (*1959*)).

Only in 1950 was Whipple's disease established as a definite entity. B. Black-Schaffer showed in 1949 that the macrophages in the jejunum and lymph nodes contained material giving a strongly positive periodic acid-Schiff (PAS) reaction (*Proc. Soc. Exp. Biol. Med.* **72,** *225* (*1949*)). This at one time provided a histologic criterion for diagnosis and suggested that the disease had a different pathogenesis from the abnormality of fat metabolism formerly considered. For this reason the term "intestinal lipodystrophy" seems less desirable than "Whipple's disease."

Farnan accepted a total of 60 cases from the literature and added seven in his report. He found the cases confined to the white race; 55 of 67 were male. The average age of onset was 39 years and the disease was invariably fatal until the advent of steroid therapy, which causes temporary remissions. (Some probable cures have been reported.) The characteristic PAS-positive material has been found in "widespread sites in a minority of cases," *e.g.* retroperitoneal tissues, liver, spleen, peripheral lymph nodes, and even in heart valve vegetations and the central nervous system.

R. H. Puite and H. Tesluk (*Am. J. Med.* **19,** *383* (*1955*)) confirmed the wide distribution of the characteristic PAS-positive material, found it in peripheral lymph nodes, and proposed biopsy of such nodes as a diagnostic procedure. The experience of others has confirmed the value of their findings.

Thus, it appears that the distinctive histologic feature of Whipple's disease is not the accumulation of fats and fatty acids, but the widespread occurrence of macrophages containing granular PAS-positive material. This may well be a glycoprotein, but its chemical nature is not further established. Its source is not proven, though many believe it arises in or enters through the intestinal mucosa.

A. S. Cohen, E. M. Schimmel, P. R. Holt, and K. J. Isselbacher have reported their studies on "Ultrastructural Abnormalities in Whipple's Disease," under this title (*Proc. Soc. Exp. Biol. Med.* **105,** *411* (*1960*)). Their material consisted of a jejunal biopsy from a 65 year old man with known Whipple's disease (*New Engl. J. Med.* **262,** *572* (*1960*)). Sections were prepared for light and electron microscopy. Many macrophages filled with PAS-positive particles were present in the lamina propria. These inclusions, when identified in electron micrographs, were complex and varied, consisting of "series of closely packed membranes, granules, and vesicles"; "tubules" were also seen, as well as ill-defined and amorphous structures.

The ultrastructure of the macrophage is not well documented. However, Cohen's group points out that laminated structures have been observed by others in various types of phagocytosed material. They believe, nevertheless, that ". . . the present large inclusion bodies with their sinuous

membranes and granules are a unique finding . . ." They also point out that it is not clear whether the inclusions consist of ingested material or of cell products.

Certain dense bodies were found just outside the macrophage cell borders, but could not be identified. These were round, oval or rod-shaped with a small pale core surrounded by dense material and a separate clearly visible outer limiting membrane, well shown in the electron micrograph reproduced in the article. The authors think that these bodies may represent material being absorbed, but point out their resemblance to virus particles.

The microvilli of the jejunal epithelium were of normal appearance in contrast to the decrease in size and number or even absence which has been reported in sprue.

Findings similar to those of Cohen, Schimmel, Holt, and Isselbacher have also been obtained by W. S. Haubrich, J. H. L. Watson, and J. C. Sieracki (*Gastroenterology* **39,** *454* (*1960*)). These authors observed sausage-shaped bodies 0.8 to 1.7 microns long within and outside of the characteristic macrophages. They also found the intestinal epithelium, including the microvilli, normal.

The return of the microvilli of the intestinal epithelium toward normal when gluten is eliminated from the diet of patients with nontropical sprue (H. Zetterqvist and T. R. Hendrix, *Bull. Johns Hopkins Hosp.* **106,** *240* (*1960*)) has suggested that their absence in some way causes a malabsorption syndrome. If the biopsy in the present case is representative, malabsorption can evidently occur with normal microvilli.

It seems likely that the cause of Whipple's disease lies with the origin of the PAS-positive material. Whether this causes or results from malabsorption, whether it is of intestinal or widespread origin, whether it is phagocytosed or produced by the cells which contain it, and what the significance is of its ultrastructure, we do not know. Although Cohen and his colleagues leave many questions unanswered, their report further documents our knowledge of the structural changes in Whipple's disease.

Increasing numbers of cases of this condition are being recognized and studied under the electron microscope. These reports will document the changes more fully and, one hopes, elucidate the cause of this interesting disease of which we have no experimental counterpart.

TOTAL BODY WATER IN MALNOURISHED INFANTS

Malnourished Jamaican infants studied with tritium initially and during therapy, showed increased total body water, markedly decreased body solids, and replacement of water by tissue without weight gain during recovery.

The majority of deaths among infants with chronic malnutrition in underdeveloped countries occur within 48 hours of hospital admission and are associated with gross disturbances of fluid and electrolyte balance. Analyses of blood and muscle biopsies (F. Gómez *et al.*, *Pediatrics* **20,** *101* (*1957*); S. Frenk *et al.*, *Ibid.* **20,** *105* (*1957*); G. Gordillo *et al.*, *Ibid.* **20,** *303* (*1957*); and J. Metcoff *et al.*, *Ibid.* **20,** *317* (*1957*) have revealed hypotonic body fluids and intracellular overhydration even in clinically underhydrated patients.

To obtain a quantitative estimate of hydration in infants with and without edema at various stages of recovery from malnutrition, R. Smith (*Clin. Sci.* **19,** *275* (*1960*), working in Jamaica, measured total body water (T. B. W.) with tritium in 24 patients.

Twenty microcuries of tritium per kg. body weight in sterile water were administered parenterally. The tritium was meas-

ured in urine by the method of J. E. S. Bradley and D. J. Bush (*International J. Applied Radiation and Isotopes* **1,** *233* (*1956*)) and Bradley, D. Davidsson, I. MacIntyre, and A. Rapoport (*Biochem J.* **62,** *34P* (*1956*)). The calculation of T. B. W., based on the highest observed concentration of tritium in urine, usually four to six hours after injection, was as follows:

T. B. W. = (Amount of tritium injected) − (Amount of tritium lost from body in time T) ÷ (tritium concentration in urine at time T). In a few selected cases, parallel measurements made in plasma samples indicated that the peak urinary level of tritium is approximately equal to the serum concentration after equilibrium and therefore provides a valid datum for the calculation. The amount of isotope lost from the body by all routes—kidney, lungs and skin—was estimated as half the product of water loss (taken to be equal to fluid intake during the six hour test period during which weight changes were negligible) and maximum urinary concentration. Tritium loss thus calculated averaged 1.8 per cent of the injected dose. In most patients, T. B. W. was measured two or three times during recovery.

For descriptive purposes, the clinical course of recovery is divided into three stages: a) the stage of edema, lasting as long as three weeks; b) the stationary phase within 35 days of the loss of edema, in which there is little or no weight gain although there is apparent improvement in the general condition; and c) the stage of rapid weight gain more than 50 days after loss of edema.

Repeated measurements were made in 17 infants and single measurements in seven. T. B. W., expressed as per cent of body weight at different stages during recovery, was 84.5 ± 1.5 per cent during stage one; 73.1 ± 2.7 per cent during stage two; and 62.6 ± 2.3 per cent during stage three. For comparison, T. B. W. in the normal, "ideal" infant over six months of age is assumed to be 60 per cent of body weight (B. J. Friis-Hansén, M. Holiday, T. Stapleton, and W. M. Wallace, *Pediatrics* **7,** *321* (*1951*)).

In the edematous stage, the high T.B.W. values could not be correlated with the degree of clinical edema. Detection of clinical edema is dependent on complex underlying factors (D. C. Darrow and S. Hellerstein, *Physiol. Rev.* **38,** *114* (*1958*)), one of which is the amount of subcutaneous fat present (Frenk *et al.*, *Pediatrics* **20,** *105* (*1957*)). It is therefore not surprising that one marasmic infant with minimal edema of the ankles only and gross loss of subcutaneous fat had as much body water as a classical case of kwashiorkor with edema and well-preserved subcutaneous fat. With respect to the edematous stage in general, two distinct but related abnormalities were revealed, first, in the *proportion* of water in the body, and second, in the *amount* of total body solids. The proportion of water in comparison with that of normal infants of the same weight is increased. In 11 out of 17 cases, the total body solids, calculated as (body weight in kg.) − (body water in liters), were less than 30 per cent of the normal or ideal for a given age, calculated from the T.B.W. data of Friis-Hansén, Holiday, Stapleton, and Wallace. This deficit in solids (averaging 19.2 per cent of normal) exceeded by far the deficit in edema-free body weight (averaging 52.4 per cent of normal). Hence, while the deficit in edema-free body weight is a convenient index of the degree of malnutrition, it grossly underestimates the severity of tissue depletion.

In the stationary stage, on the average, T.B.W. was still increased after disappearance of clinical edema. This may explain why weight gain is slow or absent in this phase despite retention of large amounts of nitrogen (J. C. Waterlow and V. G. Wills, *Brit. J. Nutrition, in press*), since it has been suggested (C. Gopalan, P. S. Venkatachalam, and S. G. Srikantia, *Metabolism* **2,** *335* (*1953*) and Venkatachalam, Srikantia, and Gopalan, *Indian J. Med. Res.* **43,** *511* (*1955*)) that the loss of excess water marks

the deposition of tissue. Whether this excess water is extracellular or intracellular is presently uncertain.

In the recovery stage, T.B.W. values moved on the average toward those for normal infants. However, in some infants T.B.W. increased following an initial fall. To investigate this paradox, Smith measured skinfold thickness and limb circumference at intervals (K. L. Standard, V. G. Wills, and J. C. Waterlow, *Am. J. Clin. Nutrition, in press*). These serial measurements, which give rough estimates of relative changes in muscle and skin (mainly fat) mass, were correlated with changes in T.B.W. In recovery, a steadily declining percentage T.B.W. was associated with a greater increase in fat than in muscle; however, a slight rise in percentage T.B.W. following an initial fall was associated with a greater gain in muscle than in fat.

The average increase in body solids over the period of observation was 27 g. per day (range −10 to +53) in 17 infants on whom initial and final total body solid values were calculated. This increase is high when compared to the normal rate for well infants of the same age of 6 g. per day. Confirmation of the authenticity of these gains is provided by nitrogen balance studies (Waterlow and Wills, *Brit. J. Nutrition, in press*) which have revealed three times the normal rate of nitrogen retention during recovery of malnourished infants on moderate protein intakes.

Smith's work is an excellent example of the synthesis of modern technology with clinical observations such as to cast new light on problems which have not yielded to conventional methods of nutrition research. More such syntheses both in the clinic and in the field are essential if progress in human nutrition research is to match progress in other medical disciplines.

RESULTS OF TREATMENT IN PHENYLKETONURIA

On the basis of published reports, treatment early in infancy with a low phenylalanine diet brings about a definite improvement in mental ability.

Some years have passed since the introduction of specific dietary therapy in the management of infants and children with phenylpyruvic oligophrenia. This disease—a classic prototype of the group known as "inborn errors of metabolism"—is due to an enzymatic deficiency in the conversion of phenylalanine to tyrosine. A number of clinical symptoms occur; among the more devastating are high grade mental deficiency and convulsions. It was with great interest, therefore, that the early reports of effective dietary therapy were received.

The fact that dietary therapy—basically a diet low in phenylalanine—does help in certain cases of phenylpyruvic oligophrenia appears well established. Recently, W. E. Knox (*Pediatrics* **26,** *1 (1960)*) has undertaken a review of reported cases in order to ascertain the degree of improvement to be expected from such a treatment regimen and to compare the fate of these patients with the natural history of the disease. The authors' personal experience (D. Y. Hsia, Knox, K. V. Quinn, and R. S. Paine, *Pediatrics* **21,** *178 (1958)*) with the treatment of this disease is included.

For analysis, the reported cases were divided into two groups: those in whom treatment was begun when the patient was under 36 months of age, and those first given the diet after this age.

There was little or no improvement in the latter group, comprised of 44 patients. Formal psychometric testing revealed no significant changes in mental performance. However, some authors reported a decrease in seizure frequency, decreased irritability

and restlessness, and improved attentiveness. The over-all picture was disappointing.

The body of Knox's report is concerned with the younger infant, for it is in this age group that the best results are to be expected. He was able to find reports suitable for analyses of some 43 patients. Ages at onset of therapy ranged from ten weeks to 36 months; age at the last follow-up evaluation from five months to six and one-half years. The mean duration of treatment was 17 months. Data on seizure status, electroencephalographic findings, degree of "chemical" control (as evidenced by blood phenylalanine concentration), and results of intelligence tests are also included.

The first question to be asked is the fate of children who have never received dietary treatment. A total of 466 such cases have been reported. Approximately 64 per cent have an intelligence quotient of 20 or below, 33 per cent an I.Q. between 21 and 60, 1.9 per cent between 61 and 80, and only 0.6 per cent reach the level of 81 or greater. The highest reported I.Q. of 103 was achieved by only one patient.

By comparison, the treated patients have done much better. Of 23 patients in whom treatment was begun prior to age 16 months, 69 per cent achieved an I.Q. above 61, and 56 per cent an I.Q. above 81. In those 20 cases wherein treatment was begun in the interval between 16 and 36 months, the figures for these intelligence groupings were 15 and 5 per cent respectively.

The effect of early treatment is graphically portrayed by plotting final achieved intelligence against age at start of treatment for all 43 patients. The correlation coefficient was calculated to be -0.67; the slope of the regression line was calculated to be -0.45. The latter value suggests that there is a loss of 0.45 points in the I.Q. for each week that treatment is delayed.

Data on other neurologic manifestations, such as EEG records and presence or absence of seizures, are not so complete. From the evidence available, it would appear that abnormal EEG records were obtained twice as frequently in untreated as in treated patients. Seizures were absent in patients treated before 16 months of age, in contrast to an incidence of 26 per cent in the untreated group. However, the incidence was high in the remaining treated patients, and the accuracy of reporting this symptom was admittedly poor.

The author concludes that early treatment of patients with phenylpyruvic oligophrenia is accompanied by a significant degree of improvement in mental and neurologic status. Furthermore, the degree of improvement is inversely related to the age at which treatment is started.

One hesitates to detract in any way from these salutary conclusions, for the helplessness which is felt in dealing with children irrevocably damaged by this disease is all too vivid. Any improvement, no matter how slight, would be welcome. That improvement occurs in some patients cannot be questioned. The point concerns the frequency with which improvement can be expected to occur and to what degree.

There is no way of knowing how many treated patients remain unreported in the medical literature. This reviewer has personal knowledge of three such patients. No doubt physicians, in general, are more inclined to report situations involving a favorable outcome and in this sense a compilation such as Knox's must perforce suffer from bias.

Knox points out that the other source of bias is the status of the untreated patient. Most of the data on this group are drawn from mental institutions and include only a small number (0.6 per cent) of "high level" defectives. Two such individuals (or 5 per cent) were included in the treated group surveyed by Knox; there is no way of knowing how many more there may be in the noninstitutionalized population at large. In eight of the patients treatment was begun before eight weeks of age or at a time

before symptoms could be expected to occur.

Granting the conclusion that early treatment in this condition is most beneficial, the matter of early diagnosis becomes of paramount importance. The difficulty here is that clinical symptoms usually do not appear before six months of age. Siblings of known patients (the disease is inherited as a Mendelian recessive) can of course be observed closely from birth and treatment instituted as soon as chemical evidence of the disease can be obtained. Usually the blood phenylalanine level rises and phenylpyruvic acid appears in the urine by the age of two to four weeks.

For other individuals without a family history of the disease, the only recourse is that of mass screening techniques in an attempt to find phenylpyruvic acid in the urine of otherwise healthy infants. Since the incidence of the disease has been estimated to be one in 20,000 to 40,000 people, this becomes a formidable task (see editorial comment, *Yearbook of Pediatrics, 1960-61 Series, Yearbook Publishers, Chicago, p. 66*). The results of screening programs now under way in certain areas of this country will be awaited with interest.

PROTEIN LOSING ENTEROPATHY

Hypoproteinemia was present in a patient who had a long section of the small intestine permeable to both albumin and globulin.

The level of protein in the blood is determined by both the rate of protein synthesis and the rate of protein degradation, providing that there are no abnormal pathways for loss of protein, such as bleeding or increased permeability of the blood vessels. Idiopathic hypoproteinemia has been reported in certain individuals where there is no edema and no proteinuria. Such patients have abnormally low serum protein levels, particularly in the albumin fraction. It has been reported earlier (*Nutrition Reviews* **17,** *261* (*1959*)) that two such patients were losing abnormally large amounts of protein into the gastrointestinal tract due to giant hypertrophy of the gastric mucosa. The use of polyvinylpyrrolidone (PVP) labelled with radioactive iodine made it possible to demonstrate the presence of abnormal permeability of the gastrointestinal tract. Since polyvinylpyrrolidone has a large molecular weight and is not digested by intestinal secretions, its determination in the feces following an intravenous injection is a good measure of permeability of the gastrointestinal wall to protein.

Recently, S. Jarnum and V. P. Petersen (*Lancet* **1,** *417* (*1961*)) studied a 49 year old woman with a protein losing disorder resulting from a diffused intestinal lesion involving a large part of the jejunum. The patient's total serum protein usually ran between 4.0 to 4.8 g. per 100 ml. with the albumin varying between 40 and 50 per cent of the total protein. In addition to the low albumin level the values for gammaglobulin, as determined by paper electrophoresis, ranged from 10 to 15 per cent of the total serum protein, which is about one-half of the normal concentration. Protein was not being lost in the urine and both her kidney and liver function tests were normal. Also, no abnormality in serum electrolytes was noted. Initial x-rays of the stomach, small intestine, and colon revealed no abnormalities, though four and six months later repeated x-rays indicated some patchy abnormalities in a limited section of the small intestine.

The patient was given intravenously polyvinylpyrrolidone tagged with radioactive iodine and the accumulative five

day loss in the feces was determined. Normal individuals, those with intact gastrointestinal tracts, usually lose less than 1.0 per cent of the injected activity during the five day period. This patient, however, lost 5.9 per cent of the administered radioactivity in the feces. This is a rather clear demonstration that there is an abnormally high gastrointestinal permeability to a large weight molecule.

To determine the albumin metabolism, this patient received serum albumin tagged with radioactive iodine. Her serum albumin level was 1.88 g. per 100 ml. with the normal values ranging from 3.8 to 5.1. The circulating albumin was found to be 0.78 g. per kg., while in normal individuals it ranged from 1.3 to 3.0. Both of these values are considerably below those for normal individuals. Twenty-six to 28 per cent of the circulating albumin was degraded per day for this patient while only 6 to 14 per cent per day was degraded by normal individuals. The albumin turnover rates expressed as g. per kg. per day were similar in both the patient and the normal individuals (0.204 for the former and 0.13 to 0.28 for the latter group). Thus, this individual was turning over her albumin at a normal rate in spite of the greatly decreased total albumin pool. This patient also lost radioactive iodinated albumin in the feces at a greater rate than did normal individuals. The five day accumulated fecal radioactivity was 2 per cent of the injected dose, as compared to the value of less than 0.3 per cent in the normal individual over a comparable period of time.

Because of the evidence of a limited lesion in a series of x-rays, a laparotomy was performed in an attempt to remove the abnormal section of the intestine. It was found that approximately 1.5 meters of the jejunum was thickened, congested, and edematous. Also numerous small brownish pigmentation areas were scattered over the entire intestinal surface. The defect was not sharply defined, but extended with gradual lessening about halfway down the intestine. Small blood vessels of the intestine were dilated and there were also numerous dilated lymphatics. These investigators decided that due to the length of the affected part of jejunum a resection of this area was not practical. Studies were carried out concerning the nature of this yellowish-brown pigment, and it was found that the material stained with Sudan black, was acid fast, and positive to periodic acid Schiff reagent. The pigment also fluoresced with a yellow light in the ultra-violet. Besides a negative staining reaction for iron, no other data were given as to the nature of this material.

By measuring the amount of radioactivity in the pleural fluid almost daily for 12 days following the laparotomy, it was found that the specific activity of the pleural fluid did not equal that of the plasma until 12 days after the administration of the tagged albumin. On the other hand, the radioactivity and the protein content of the ascitic fluid exchange rapidly with the plasma. These investigators suggest that the hypoproteinemia in this patient is due to an abnormal transfer of plasma protein into the intestinal lumen and the peritoneal cavity.

RADIO TOXICITY OF IODINE^{131} IN DAIRY CATTLE

A level of 100,000 rad needs to be applied to the bovine thyroid before radiation damage will result in general systemic effects that can be observed. Reproduction was essentially normal in animals with damaged thyroids, but milk production was drastically reduced.

The Windscale incident, with its consequent release of iodine^{131} into the atmosphere and the resultant deposition of iodine^{131} on forage in the vicinity, raised serious

questions with respect to the level of iodine^{131} that would cause detectable injury in dairy cows. The detection of radioiodine in the milk of cows grazing on the area surrounding the reactor was reported early, and eventually, permissible levels which might be tolerated in the milk under the emergency conditions were set up by the responsible authorities.

When the incident occurred in 1957, there was very little information on radiotoxicity in livestock available except for the work of L. K. Bustad *et al.* (*U.S.A.E.C. Biol. Res. Ann. Rep. Doc. No. HW35917, p. 129 (1955)*) which had been carried out with sheep. There is considerable question as to the validity of extrapolation of results with sheep directly to the cow.

Directly as a result of the Windscale incident and in an effort to obtain fundamental information on the radiotoxicity of iodine^{131}, R. J. Garner, B. F. Sansom, H. G. Jones, and L. C. West (*J. Comp. Path.* **71**,*71 (1961)*) have reported a small-scale experiment using Ayrshire-Red Poll dairy cattle. These mature cattle were selected for experiment during their first month of lactation; they were chosen during different periods of the year so that a study of the seasonal effect on thyroid activity might be included in the experiment.

At least four animals were used for each of the levels of radioactive isotopes administered. The analysis of the diets provided these animals showed that at the beginning of the experiment 3,000 μg. of iodine were contained in the daily ration of each cow. Somewhat later, due to a change in the commercial diet, the iodine level was raised to 7,000 μg. per day per cow. This compares with the approximately 400 to 800 μg. per day which may be considered adequate for the daily requirement of a mature dairy cow.

In designing their experiment these investigators attempted to simulate the Windscale pattern as nearly as possible under controlled conditions. They selected levels of 1.4, 14, and 140 mc. of iodine as the dose levels in an effort to supply amounts of iodine^{131} which would cause no damage, minimal damage, and severe damage to the thyroid gland. In order to simulate the conditions on pasture such as would occur if iodine^{131} were released to the atmosphere, the iodine^{131} was administered in 14 equal, consecutive, daily doses. Later, as a result of information gained during this experiment, levels of 0.5, 2.0, and 5.0 mc. per day were also used.

In the course of the experimental work these investigators discovered that a number of factors influenced the level of iodine^{131} absorbed and accumulated in the thyroid gland. One of the most important factors was the level of stable iodine present in the diet. The increase from 3,000 to 7,000 μg. per day markedly reduced the absorption and accumulation of the radioactive iodine in the thyroid gland. In addition to the dietary factors, there was a marked individual animal variation in the fraction of the iodine^{131} dose which went to the thyroid gland. This ranged from 7 to 36 per cent of the level administered.

The seasonal effect was also quite marked and was attributed to the well-known variation in thyroid activity between winter and summer.

The authors had attempted to select a dose of iodine^{131} which would provide different degrees of damage and chose 1,000, 10,000 and 100,000 rad. (A rad is a unit of absorbed energy and is equivalent to the absorption of 100 ergs of energy per gram of tissue.) In selecting these levels and translating them into terms of millicuries of iodine^{131} certain basic assumptions had to be made which later proved to be in error. This led to somewhat different amounts of radiation being accumulated in the thyroid glands of the animals.

Due to the variations in animal absorption of the administered dose, subsequently determined differences between actual and assumed thyroid weights, and the thyroid

activity reflecting the season of the year, the levels of radiation in the thyroid actually ranged from 1,500 to 290,000 rad.

The levels above 70,000 rad caused destruction of functional thyroid tissue. Since the next higher level was 150,000 rad, it was possible to say that all the levels used in this experiment above 70,000 rad caused complete destruction of the functional thyroid tissue. The clinical symptoms which appeared as the result of this destruction were essentially those of thyroidectomy.

It was apparent from blood plasma bound iodine (PBI) evaluation that the storage capacity of the thyroid gland was affected at an early stage in iodine^{131} administration. Evidence of this was observed before all of the iodine^{131} was administered or when approximately 75,000 to 150,000 rad had been accumulated in the gland. Except for a slight swelling over the thyroid which disappeared in relatively short time, the changes in the animals receiving the levels of 70,000 rad and above were quite minimal.

At two months, when functional tests were made of the thyroid of these animals, it was observed that the animals receiving the highest level of iodine^{131} were quite lethargic and in some cases had an edema of the legs; all of this group showed a reduction in milk flow. Appetite was not seriously affected; the reduction in storage capacity of the thyroid gland appeared to be the most outstanding observed change in these animals.

It was obvious from the results of Garner, Sansom, Jones, and West that the thyroid of the cow is much less sensitive to radiation damage than is the thyroid of the sheep, in which Bustad *et al.* showed that extensive necrosis occurred by administration of 33,000 to 75,000 rad. In the cattle a total radiation of 70,000 rad administered to the thyroid appeared to have but a slight and very transient effect, which was not detectable by a thyroid function test two months following the administration of the iodine^{131}.

It was noteworthy that the parathyroid was apparently not affected by this destruction of the thyroid gland with the highest levels of iodine^{131}, since an evaluation of calcium and phosphorus concentration indicated that there was no change.

Since the thyroid is so intimately concerned with reproduction in most species, an attempt was made by Garner, Sansom, Jones, and West to evaluate the effect of thyroid exposure to radiation damage upon reproduction. Estrus appeared to be normal. The animals were bred without difficulty and carried their calves to term with normal calves being born. It is worthwhile pointing out that in the animals which had evidence of thyroid destruction, some difficulty was experienced in calving. This follows the general pattern observed in thyroidectomy.

It may be pointed out that the thyroid destruction appeared to produce no diminution in fertility and, as is the case in thyroidectomy, radio-thyroidecraxis (thyroid destruction by radioiodine) did not prevent the birth of normal calves. The thyroid glands of these calves were quite normal.

The effect of iodine^{131} on the thyroid gland was especially noticeable with respect to lactation. All of the animals which received 140 mc. of iodine^{131} during the first month of lactation had ceased lactating within 135 days with a corresponding reduction in milk yield. It may well be pointed out that individual variation was apparent even with the highest level of administered iodine^{131}. One of the four animals on the 140 mc. level did not show complete destruction of the thyroid and evidenced recovery of thyroid function within the period between administration of the radioactive iodine and the initiation of lactation following the birth of a calf.

The report of Garner, Sansom, Jones, and West indicates that a radiation dose of at least 100,000 rad is necessary to damage the thyroid gland of the lactating cows sufficiently to produce general effects in the animal. It is quite difficult to equate this to the level of iodine^{131} which must be injected to produce this effect. The variation in

thyroid activity with the season of the year and the level of stable iodine in the diet, as well as individual animal variation, prevent any accurate estimation of the amount of iodine^{131} which must be administered to result in a thyroid accumulation of 100,000 rad. It would appear, however, assuming that 20 per cent of the ingested dose would go to the thyroid, approximately 90 mc. of iodine^{131} would have to be consumed over a relatively short period of time to deliver such a radiation dose.

If one is to translate these experimental results to practical situations such as might occur with animals on pasture, one would have to assume that there would need to be an initial deposition of iodine^{131} on a pasture at a level of 240 mc. per square meter. In order to put this in perspective, it may be pointed out that the maximum permissible level of iodine^{131} in milk for human consumption under conditions which occurred during the Windscale incident was set by the British authorities at 0.065 mc. per liter. Such a level in milk is achieved by a deposition of 0.4 mc. of iodine^{131} per square meter.

Garner, Sansom, Jones, and West have made a valuable contribution to our knowledge of the radio toxicity of iodine^{131} insofar as dairy cattle are concerned. Their work emphasizes the need for continuing research with various species of animals. The rather wide variation in species sensitivity to radiation dose, the apparent seasonal and dietary factors, and individual variation all emphasize the hazards of direct extrapolation of research on radiation damage from one species to another. The need for an evaluation of radiation hazard inevitably requires extended investigations with a number of different animal species before reliable conclusions can be drawn as to the effects of different radioactive isotopes upon animals.

BOUND NIACIN

Two compounds containing biologically unavailable niacin have been isolated from wheat bran and have been partially characterized.

The occurrence of a bound form of niacin in cereal products was postulated about 20 years ago when aqueous and mild acid extracts of cereal grains were found to contain little free niacin by microbiological assay, whereas extracts of alkali-treated grains were good sources of niacin (E. Kodicek, *Biochem. J.* **34,** *712, 724 (1940)*). Subsequently, untreated cereal grains were shown to be poor sources of niacin for mammals as well as for bacteria, while alkali-treated grains stimulated the growth of niacin-deficient mammals very effectively. Kodicek (*Brit. J. Nutrition* **14,** *13 (1960)*) has recently discussed the nutritional and earlier chemical studies on the availability of niacin in cereal products.

The nature of the bound form of niacin has remained elusive and even its existence has been questioned during the intervening years. Recently, two reports, one by Kodicek and P. W. Wilson (*Biochem. J.* **76,** *27P (1960)*) and the other by M. L. Das and B. C. Guha (*J. Biol. Chem.* **235,** *2971 (1960)*) describe homogeneous and crystalline preparations, respectively, of bound forms of niacin isolated from wheat bran and from rice bran. The latter authors obtained a similar product from maize; other investigators, including Kodicek, have studied maize extensively as a source of bound niacin. Current evidence suggests that preparations of bound niacin from different cereal grains are similar although they may not be identical.

Kodicek and Wilson extracted wheat bran with 0.1 N HCl and obtained bound niacin after fractional precipitation of the extract

with acetone. The precipitate was extracted with pyridine and phenol and the final product was precipitated from the extract with acetone and 85 per cent ethanol. Using this procedure they obtained 30 mg. of a brown powder from 100 g. of wheat bran.

Das and Guha used the same initial steps but substituted an acetone-ether (2:1) mixture for acetone in the fractional precipitation. They dissolved the precipitate in 65 per cent ethanol and reprecipitated with the acetone-ether mixture. The precipitate was dried, dissolved in water, and passed through a cellulose column which was developed with phenol-water (80:20) as the solvent system. The bound niacin, which was not held on the column, was precipitated from the eluate with acetone-ether, was dried and redissolved in a small amount of water. It crystallized from the final solution as hygroscopic, brownish, rectangular plates. The yield was 60 mg. per 100 g. of wheat bran.

The product obtained by Kodicek and Wilson yielded on hydrolysis the following components in per cent: niacin, 5; glucose, 48.5; xylose, 8; arabinose, 4; an aromatic amine, 5; phenolic esters (substituted cinnamic acids), 27; and protein impurity, 2. The product was homogeneous by various measurements including electrophoretic studies. It had a molecular weight of about 2,400 based on the empirical formula and niacin content.

The product obtained by Das and Guha was less well characterized. The percentage composition can be calculated roughly from the information given, as niacin, 1; amino acids, 75; glucose, 1.5; unidentified chromogens to make up the remainder. Evidence for homogeneity of the material is not as satisfactory. Three preparations from different starting materials behaved the same chromatographically in three solvent systems, but in two they did not move from the origin and in the third they moved with the solvent front. Repeated crystallization gave the same kind of crystals. On ultracentrifugation only a single component was seen. From diffusion experiments at pH 2 the molecular weight was estimated to be between 12,000 and 13,000 which agreed with the value of 12,300 calculated from the niacin content of 1 per cent. Diffusion and ultracentrifuge measurements at pH 8 yielded values closer to 6,000 which the authors explain by assuming that the molecule dissociates into two approximately equal halves at alkaline pH.

The question arises as to whether one or the other or neither of these compounds is the naturally occurring form of bound niacin. Das and Guha found no arabinose or xylose in their compound and suggested that at least part of the chromophore was a flavonoid. Most of the molecular weight of their substance was made up of amino acids. Interestingly, they found the amino acid composition of compounds isolated from rice and wheat to be similar, although not identical. Kodicek and Wilson considered the small amount of polypeptide in their compound to be an impurity and identified xylose, arabinose, phenolic esters, and an amine as components as well as glucose. The molecular weight of the nonpolypeptide portion of the compound isolated by Das and Guha would be somewhat larger than, but of the same order of magnitude as, the compound isolated by Kodicek and Wilson. Most of the nonpolypeptide portion of the compound isolated by Das and Guha remains unidentified but they detected no arabinose or xylose among the hydrolytic products. The possibility that the different methods of isolation result, in one case, in the removal of some of the carbohydrate components and, in the other, of the protein to which the niacin or niacin-containing complex is bound, seems quite possible.

Kodicek and Wilson observed that among the products of partial hydrolysis with N HCl they obtained one composed only of niacin, an aromatic amine, and two molecules of glucose. Even this relatively simple compound could not substitute as a source

of niacin for *Lactobacillus casei*. It was apparently not obtained in sufficient quantity to test as a source of niacin for the rat. These results suggest that regardless of the complexity of the naturally occurring product, only the direct linkage, apparently an ester linkage through the carboxyl of the nicotinic acid, is required to make niacin unavailable. The linkage was split very slowly by liver esterase, according to Kodicek and Wilson. It must be split little if at all by gastrointestinal enzymes. Even if the naturally occurring compound is some type of chromoprotein containing carbohydrate and aromatic moieties, it would seem a safe assumption that most of the amino acids are removed during digestion and that some characteristic of the ester-linkage of the nonprotein portion is responsible for the lack of availability of niacin from cereal products.

These papers by Kodicek and Wilson and by Das and Guha, represent strides toward elucidation of the nature of the bound form of niacin. However, since the products isolated in the two laboratories are obviously distinctly different and since neither product has been completely characterized, they do not provide a final solution to this intriguing problem in vitamin chemistry.

POTASSIUM DEFICIENCY AND THE KIDNEY

Prolonged potassium deficiency results in impairment of renal function and in histological abnormalities of the renal tubules.

Some years ago, clinicians used to speak of the "alkalotic kidney." This term referred to the changes in renal function noted in patients suffering from metabolic alkalosis caused by prolonged vomiting. Since these patients usually had symptoms of dehydration and malnutrition in addition, there was some question as to the origin of the renal functional damage. Not all observers were ready to claim that the metabolic alkalosis was solely at fault.

In fact, repair of the dehydration in such patients was usually associated with a return to normal of renal function. However, R. Rohland (*Klin. Wchnschr.* **15,** *825* (*1936*)) described "hypochlorämische Nephrose," and later C. H. Burnett, B. A. Burrows, and R. R. Commons (*J. Clin. Invest.* **29,** *169* (*1950*)) found that a degree of impairment of renal function persisted for many months in some patients, despite the initial postdehydration improvement.

During this same era, it became evident that patients suffering from hypochloremic metabolic alkalosis were also depleted of potassium. In recent years, the consensus is that the nephropathy of metabolic alkalosis has its origin in potassium deficiency.

Descriptions of "nephropathy of potassium depletion" have been made by A. S. Relman and W. B. Schwartz (*New Engl. J. Med.* **255,** *195* (*1956*)) and of the defect in renal concentrating ability by W. Hollander, Jr. *et al.* (*Am. J. Physiol.* **189,** *557*, (*1957*)). Certain aspects of this problem have been discussed previously (*Nutrition Reviews* **13,** *148* (*1955*); **14,** *331* (*1956*)).

In a recent article, M. A. Holliday *et al.* (*Pediatrics* **26,** *950* (*1960*)) describe their experiments bearing on this subject. Rats were rendered deficient in potassium by dietary means, and were observed for evidences of renal abnormalities. The presence of potassium deficiency was confirmed by analysis of muscle; in some experiments, particularly those in which dietary sodium was excessive, muscle potassium was only 70 per cent of normal. Histological studies of kidney tissue were done, and tests of the ability of the kidney to concentrate and to dilute the urine were carried out.

No effect was found as far as the ability of the kidney to elaborate a dilute urine was concerned. Potassium deficient rats excreted water loads in normal fashion, and a minimum urine osmolarity of approximately 90 milliosmols per liter was achieved.

Response to combined water deprivation and vasopressin administration became progressively impaired as the period of potassium depletion was lengthened. The tests were carried out by fasting and thirsting the animals for 24 hours. Vasopressin was given at the fifth hour and urine collected from the eighth to the twenty-fourth hours. Under these conditions urine osmolarity rises to approximately 2,700 milliosmols per liter. This value declines slightly within three days, and continues to fall as depletion continues, reaching a value of 800 milliosmols per liter at the end of 114 days.

Potassium deficiency was also associated with a slight fall in glomerular filtration rate, as measured by inulin clearance, with average values of about 80 per cent of normal. Clearance of "free water" also declined slightly.

Kidney size increased by 29 per cent. Histological abnormalities occurred quickly. These typically involve the collecting tubule, with droplet formation in the distal portion, and hyperplasia and proliferation of cells in the more proximal regions. The latter often leads to tubular dilatation, some destruction of nephrons, and eventually to scarring of the kidney.

Further experiments were done in order to study the phenomenon of repair when a deficient animal was given potassium. Animals deficient for 114 days were studied on adequate potassium diets for an additional 86 days.

Within 15 days of repletion urinary concentrating ability had risen from 800 to 1,600 milliosmols per liter (normal 2,700), but no further increase occurred. Inulin clearance returned to normal. Evidences of cellular proliferation disappeared, but renal scarring persisted. It thus appears that the nephropathy of potassium depletion is only partially reversible, and the implication is that a certain amount of the renal damage may be permanent. Unfortunately, attempts to repair the lesion were made only in those animals subjected to prolonged deficiency, so that the question of reversibility in shorter periods of deficiency cannot be answered.

One further aspect of these experiments should be noted. Two groups of potassium deficient animals were studied, one on a high sodium diet, the other on a low sodium diet. No differences were found between these two groups. This is of interest in view of the well known fact that many other features of the potassium deficient state (for instance, sodium entry in muscle cells) can be accentuated by giving a high sodium diet.

In a companion article, E. Kerpel-Fronius, G. Romhányi, B. Gáti, and E. Dobák (*Pediatrics* **26,** *939 (1960)*) report similar studies in rabbits. They compared the renal functional and histological changes of sodium depletion to those of potassium depletion. Potassium depletion was produced by theobromine injections plus diet, sodium depletion by intraperitoneal dialysis.

Urinary concentrating ability was impaired in both states, as was creatinine clearance. However, the impairment was greater in the sodium depleted animals. This result is to be expected from the dehydration and circulatory impairment resulting from the procedure used to produce sodium depletion.

Histological changes comparable to those reported by Holliday *et al.* were found in potassium depletion. In sodium depletion the changes consisted of swelling and hydropic degeneration of the proximal convoluted tubules.

It is of interest that certain types of electrolyte depletion can lead to such profound impairment of both renal function and renal architecture. The damage, at

least in potassium depletion, is severe, and may be permanent. It is likely that the polyuria and vasopressin unresponsiveness seen in patients with aldosteronism or following desoxycorticosterone therapy is related to potassium deficiency.

LONG-TERM FEEDING OF FATS AND ATHEROSCLEROSIS IN CHICKS

Chicks fed rations containing either corn oil or animal fat for three years showed no differences in serum cholesterol levels or degree of atheromatous lesions in the aorta. Highest mortality was in the group fed animal fat. Those that died had higher serum cholesterol levels than the survivors.

The chick has been used extensively to study the effect of dietary lipids on blood cholesterol levels and the incidence of atherosclerosis associated therewith. Most of these studies have been continued for three to five weeks. The results confirmed the conclusions reached with other animal species over equally short-term periods and the opinions held by some of the investigators in this field (*Nutrition Reviews* **18,** *281 (1960)*). Such studies suggested "that the saturated acids tend to elevate the serum cholesterol in chicks while the polyunsaturated acids tend to lower it and the monounsaturated acid (oleic) has little effect" (*Ibid.*).

H. Fisher *et al.* suggest that similar feeding tests carried out with chickens over periods as long as three years might give different results (*Brit. J. Nutrition* **14,** *433 (1960)*). They fed white leghorn pullets a corn-soybean meal ration unsupplemented with fat until the birds were 12 weeks of age. At that time 20 birds were fed the basal diet alone, another 20 birds were fed a similar ration with 10 per cent added corn oil, and the third group was fed the same ration with 10 per cent animal fat (yellow grease, a commercial grade of animal fat). All birds were maintained in individual pens equipped with wire floors in a room maintained at a constant temperature. Over the three year period, three birds died in the group receiving the basal diet, five died in the corn oil supplemented group, and nine died in the animal fat supplemented group.

Throughout the experiment, systolic blood pressure was not influenced by the dietary supplement. At the end of the study, the blood pressure ranged from 175 (mm. of mercury), the average for the animal fat group, to 183 for the corn oil group. The plasma cholesterol values at the end of each of the three years were essentially the same for the three dietary groups. The mean cholesterol levels for the entire experimental period were 217 mg. per 100 ml. of blood for the birds fed the unsupplemented diet, 229 for those fed the corn oil diet, and 239 for those fed the animal fat diet.

The plasma cholesterol levels in the birds that died in the unsupplemented and animal fat groups were higher than in those that survived. This was based on the last blood sample secured prior to death. In the unsupplemented group one of the three hens that died had a plasma cholesterol level of 371 as compared to 202 mg. per 100 ml. for the survivors. Of the nine birds that died on the animal fat diet, six had an average plasma cholesterol level of 555 as compared to 243 mg. per 100 ml. for the survivors. However, in the group fed corn oil, none of the five birds that died had a plasma cholesterol level significantly higher than those of the survivors.

The cholesterol concentrations of the aorta were higher in the abdominal than in the thoracic segment of the aorta. This was true for all groups. There was no difference in the cholesterol level of the thoracic segment among the groups—3.2 to 3.8 per cent cholesterol in the dry, defatted tissue.

The cholesterol in the abdominal segment increased progressively from 5.5 in the unsupplemented group to 8.3 per cent in the animal fat group. There was no significant correlation between aorta and plasma cholesterol levels. These results suggest that the deposition of cholesterol in the aorta was not dependent primarily on the level of circulating cholesterol.

The aortic score was based on an examination of the tissue removed at the time of sacrifice. The score put greatest emphasis on the degree of lumen encroachment by proliferative lesions and secondarily evaluated gross lypemia, hardness, and roughness of the intimal surface. On the basis of aortic scores, the lesions were slightly more severe in the animals raised on the unsupplemented diet, as shown by scores of 1.27 for the thoracic segment and 2.54 for the abdominal. The lowest scores appeared in the aorta of the chicks fed the animal fat supplemented-diet—1.19 and 2.004 for thoracic and abdominal segments respectively. The scores for the corn oil supplemented chicks were intermediate. It should be emphasized that the scores represent lesions of a mild nature since the most severe lesion would have received a score of six.

Although Fisher *et al.* (*Ibid.*) suggested that the over-all performance favored the corn oil supplemented group, the outstanding observation is that for the survivors there are only slight and possibly insignificant differences between that group and the hens fed the animal fat. It is true that some of the birds that died on the animal fat diet had high cholesterol levels. It would appear desirable to study the few birds that do develop these elevated cholesterol levels in an effort to determine the biochemical or physiological factors responsible for these increases.

DIETARY COMPONENTS AND ACCUMULATION OF RADIONUCLIDES IN THE BODY

In this country about 39 per cent of the Sr^{90} and Cs^{137} in the diet comes from dairy products, and the remainder from other foods. In the absence of weapons testing, the proportion of radionuclides from milk will decrease and from nonmilk products will increase.

Although in addition to the short-lived isotopes, all the radionuclides may have some role in the total radioactive isotope burden of the human body, for many reasons, including half life, origin, and chemical properties, only Sr^{90} and Cs^{137} are of major concern.

Sr^{90} has been of special interest because of the close metabolic relationship between strontium and calcium, and because of the use of milk as an indicator of the level of fallout, resulting from nuclear explosions, which may be present in our human diet. The United Nations Scientific Committee on the Effects of Atomic Radiation (*General Assembly 13th Session, Suppl. No. 17, Rept. A/3838, p. 103 (1958)*) emphasizes that various steps in the pathway between fallout and deposition in the tissues of man can alter the proportion of strontium to calcium that will be accumulated. A comparison of the ratio of Sr^{90} to calcium in a feed, and the ratio in milk, for example, shows that the ratio in milk is but one-tenth that in the diet. This value, which has been relatively well established for cattle, has been recently shown by S. A. Lough, G. H. Hamada, and C. L. Comar (*Proc. Soc. Exp. Biol. Med.* **104,** *194 (1960)*) to be equally true for human beings. Similarly, there is a change in the Sr^{90} to calcium ratio from diet to bone or to muscle, with the ratio in the tissues being approximately one-fourth that in the diet.

R. H. Wasserman and C. L. Comar (*J. Agr. Food Chem.* **9**, *113*, (*1961*)) have pointed out that the comparative ratio of Sr^{90} to calcium (or as they have designated it, the observed ratio (O.R.)), while not a perfect measure of the movement of Sr^{90} from diet to tissue, is still the best measure presently available. They emphasize, referring to data published by E. P. Hardy, S. Klein, and J. Rivera (*Health and Safety Laboratory, U. S. A. E. C. Rept. HASL-88, July* (*1960*)), that about 39 per cent of the Sr^{90} in the diet in the United States has been contributed by milk and approximately 61 per cent by nonmilk foods, including drinking water. The Sr^{90} to calcium ratio in milk in the United States is about one-half to one-third that reported in vegetable sources. To account for this relationship, Wasserman and Comar have reviewed the effects of various vegetable handling treatments. If the vegetable plant and the forage consumed by the cow may be postulated to have similar contamination with Sr^{90}, then instead of a ratio of one-half to one-third, the ratio of Sr^{90} to calcium in milk should be approximately one-tenth that found in vegetables.

A review of vegetable processing indicates that much of the Sr^{90} from fallout is lost when the outer leaves of such plants as cabbage and brussel sprouts are discarded. Washing and blanching of foods in the various food processing industries also significantly reduces the Sr^{90} content of the processed foods. The milling of grains results in the discard of portions of the grain which are proportionately high in Sr^{90}. Thus, between the discrimination exerted by the bovine mammary gland and the technological barriers in the food processing pattern, man is subjected to significantly less Sr^{90} in the diet than would otherwise be the case.

R. S. Russell (*Symposium on Radioisotopes in Biosphere, Univ. of Minnesota, October* (*1959*)) has emphasized that most of the Sr^{90} in vegetation is from direct foliar contamination in contrast to root absorption, or plant base absorption. Wasserman and Comar point out that if this is the case and we have no further nuclear testing, the soil, which would be the reservoir for Sr^{90} entering the food chain, would provide approximately equal amounts to the cow and to the vegetable products consumed by man. Since processing would remove less of this Sr^{90}, it follows that under these circumstances increasingly more Sr^{90} would be absorbed by human beings from vegetable products and less, proportionately, from milk products. About 82 per cent of the Sr^{90} would come from plant sources and 17 per cent from milk, with negligible amounts from meat. Obviously, dietary habits will determine the amount of Sr^{90} which may be ingested from various sources. Interestingly enough, if present evidence can be relied on it is apparent that individuals subsisting entirely on milk would have a body burden one-third that of nonmilk drinkers; the greater the proportional intake of milk, the smaller the body burden of Sr^{90}.

Important emphasis must be placed on the high calcium content of milk, and the significant discrimination against strontium by the lactating cow. The presumption must also be made that foliar contamination will become less important the longer we continue without nuclear testing.

The other isotope of particular importance is Cs^{137}. Wasserman and Comar point out that cesium behaves metabolically somewhat like potassium, and is readily absorbed from the gastrointestinal tract. It accumulates in muscle and in other soft tissues, and has a relatively short biological turnover time. On the other hand, cesium and potassium move across cell membranes at different rates, and they have different affinities for intracellular materials. When the over-all performances of cesium and potassium are considered, it must be concluded that cesium moves somewhat independently and noncompetitively in relation to potassium.

Because of the independent handling of cesium, the Cs^{137} to K ratio is of less value than the Sr^{90} to Ca ratio. Because the level of potassium in muscle and dry weight tissue is reasonably constant from animal to animal, and therefore a Cs^{137} to K ratio in these tissues may be of value, the Cs^{137} to K ratio cannot be used in the same way that the Sr^{90} to Ca ratio is used.

An evaluation of Cs^{137} sources in the diet indicates that milk and milk products are a principal source. W. H. Langham and E. C. Anderson (*Health Physics* **2,** *30, (1959)*) show that approximately 60 per cent of the dietary Cs^{137} comes from milk products and 39 per cent from nonmilk products, with approximately 24 per cent coming from meat. Wasserman and Comar, in evaluating the metabolism of Cs^{137} in experimental animals, come to the conclusion that the relative amount of Cs^{137} in the human diet, both from milk and from nonmilk products, will not change with fallout conditions or with time.

The data developed by these investigators on the relationship of dietary components to the accumulation of radionuclides within the human body have been extremely helpful in evaluating the extent of the exposure of the human population. More particularly, these reports have emphasized that the patterns of good dietary practice remain the best guides for keeping the body burden of radioactive isotopes at a low level. Even though milk and milk products contribute a large proportion of the Sr^{90} and Cs^{137} in the diet, the net accumulation of the nuclides in the body from dairy foods would appear to be less, proportionately, than that from nonmilk products.

ACCELERATED GROWTH OF RATS

Acceleration of body weight gains in rats by restricting litter size at birth indicates that slow-growing rats never catch up. Some functions such as body length, organ weights and sexual development are related to body weight, while others, e.g. tooth and eye development are determined by age.

During the past 50 years, an increasing number of reports have shown that children are growing at a more rapid rate and that this rate is being maintained (*Nutrition Reviews* **15,** *205 (1957)*). Improvements in food and nutrition have been credited for a major share of the increased growth rate. There has, however, been no valid means of ascertaining whether the increased rate of development has been associated with any over-all improvement in intellective function or in longevity.

To study some aspects of these problems, E. M. Widdowson and R. A. McCance (*Proc. Roy. Soc.* (*London*) **B152,** *188 (1960)*) varied the rate of development of newborn rats by limiting the number of pups left with one mother and adding to those born to another. This was accomplished by mixing the rats of mothers who delivered on the same day; three pups were returned to one mother, while the second mother received from 15 to 20. The mothers accepted the animals as long as the adjustments were made during the first 24 hours after birth.

All animals were weaned at 21 days and then given a stock diet ad libitum. At this stage, each of the male rats raised in the small litter weighed 60 g. whereas the ones raised in the large litter weighed 14 g. From that time on the heavier animals continued to increase in weight at a rate which was greater than that of the other group. At 500 days of age, the fast-growing rats averaged 463 g. in weight, and the slow-growing animals 319 g. (G. Lát, Widdowson, and McCance, *Proc. Roy. Soc.* (*London*) **B153,** *347 (1960)*). These observations suggest that the rats that had been stunted during the first week of life never caught up with the

body weights of the more rapidly growing animals.

The above observations are in agreement with those of G. C. Kennedy (*J. Endocrinol.* **16,** *9* (*1957*)) in so far as the male rats were concerned. He used the same technique and found that the male rats from the small litters reached a weight of 450 g., while the ones from the large litters never exceeded 300 g. The females from the large litters reached the same weight as those from the small litters but at a later date.

Outwardly, the animals from both groups appeared normal. The accelerated increase in body weight of the rats was associated with a proportional increase in length of the body as measured from nose to anus. A plot of this body length against body weight showed a smooth curve, with the values for the fast- and slow-growing rats fitting the curve perfectly. These observations indicated that the rate of growth of the skeleton was related to body weight and not determined by age.

The weights of the kidneys, the adrenals, the liver, the stomach, and the small intestine varied with the body weight and not with the age of the animal. The weight of the heart was slightly greater in the fast-growing animals than in the slow-growing animals. This relationship was true even when animals of the same weight were compared. The weight of the spleen in the slow-growing animals was exceptionally low for the animals weighing less than 20 g. As the animals increased in size, the difference between the size of the spleen in the fast- and slow-growing animals actually became accentuated until shortly after weaning, when the spleen was approximately the same per cent of the body weight in the two groups. Widdowson and McCance (*loc. cit.*) suggested that in young rats which are undernourished there is an atrophic degeneration of the spleen, especially of the hematopoietic tissue. This might explain the small size of the spleen in the slow-growing animals prior to weaning.

The weight of the testes increased very rapidly through the fifty-sixth day for the fast-growing animals, after which there was very little increase. In the slow-growing animals, it was 77 days before the weights of the testes plateaued. There is a similar indication of an accelerated rate of sexual development in the females. The vagina in the fast-growing rats opened on the thirty-sixth to thirty-seventh day, whereas the same change occurred in the slower-growing animals ten days later. When mated with normal males, the fast-growing females became pregnant in the eleventh week of life, whereas the slow-growing females became pregnant in the seventeenth week.

The rate of development of such organs as the teeth and the eyes was related to chronological age more than to body weight. This was shown by the fact that the lower incisors erupted practically at the same time for the two groups of animals. There was only one day's difference in the time when the eyes opened.

There was no difference in the number of pups in the litters born to the two groups, and the average weight of the young was the same in both cases. The latter was true for both the newborn and the weanling rats—in these cases, the litters remained undisturbed.

One of the more prominent biochemical changes observed between the two groups was the marked increase in per cent of body fat during the early stages of growth. In the fast-growing rats body fat increased to 15 per cent when the body weight was 50 g. The percentage of body fat rapidly dropped to about six per cent when the animals weighed 100 g. The slow-growing rats did not show this peak in the per cent of body fat; they showed a gradual increase in body fat and joined the curve of the fast-growing rats at about 150 g. of body weight.

The fat-free body weight of the fast-growing rats was at all ages greater than that of the slow-growing animals. At 160 days, the fast-growing rats weighed 390 g.

and had 290 g. of lean body mass. The slow-growing rats weighed 270 g. and had 240 g. of lean body mass.

The skeletal development of the fast-growing animals, when measured by the nose to anus length, was related to body weight regardless of chronological age. The long bones, however, did not lengthen as rapidly as the body (J. W. T. Dickerson and Widdowson, *Proc. Royal Soc.* (*London*) **B152,** *207* (*1960*)). This meant that the fast-growing rats had femurs which were short for their body weight and body length.

From a chronological standpoint, the fast-growing rats showed an acceleration in ossification of the forepaws. However, when the fast-growing rats were compared with slow-growing animals of the same body weight, the latter showed more ossification centers in their metatarsals. In other words, although the rate of skeletal development was related to body weight, ossification was actually more advanced in the slow-growing rats when they were compared with animals of the same body weight.

Additional support for the above came from the observation that for animals of comparable body weight, the femurs of the slow-growing rats had a higher percentage of calcium than those of the fast-growing animals. This was true throughout life. A similar situation existed in so far as the calcium content of the whole body was concerned.

Evidence was presented by Lát and Widdowson (*loc. cit.*) which indicated that the fast-growing rats were physically more active. The rats were tested by being placed in a wooden box with two compartments separated by a door which could be opened by remote control. In this situation the animals could be observed unobtrusively. The fast-growing rats moved about, cleaned and groomed themselves more frequently, and moved through the door more rapidly after it was opened, than did the slow-growing animals. This was true for all ages studied up through the forty-ninth day. At that time, the fast-growing animals weighed 166 g. and the slow-growing animals 128 g. No additional observations were made until the five-hundredth day. At that time, when one group weighed 463 g. and the other 319 g., the activity of both groups was essentially the same.

Since these investigators found that when animals from the two groups weighing the same but of different ages were compared, their activities were approximately the same, they suggest that nervous activity is more closely related to physical development than to age.

Although no evidence was secured on learning, the investigators suggested that "Had it been possible to test the speed of learning of these fast-growing and slow-growing animals more directly and by means of more precise methods, it is probable that the fast-moving ones would have been found to learn to solve their problems more rapidly. . . ." It is this area that should elicit additional work and investigation. A number of investigators noted in the above paper have indicated that the brighter children in a school were, on an average, taller and heavier than the less bright even though their chronological ages were the same. It has frequently been claimed that both the intellective and the physical developmental differences are a reflection of genetic variations. Experiments such as the above permit a control of the genetic background and offer a valuable tool for the investigation of the relation of learning capacity to physical development.

The above work is important in emphasizing the fact that the development of certain physiological processes is probably related to the physical development of the animal, whereas other reactions occur at a chronological date regardless of the stage of physiological development. It would be extremely interesting to learn the mechanism which control these differential rates of development. First, it would be important

to establish that the observations recorded by Widdowson and her co-workers are characteristic of strains of rats other than the unnamed one used by them.

REGULATION OF CHOLESTEROL METABOLISM WITH THYROXINE ANALOGUES

The addition of 3,5-diiodothyroacetic acid to an atherogenic diet inhibited hypercholesterolemia and reduced the concentration of cholesterol in the liver of rats. The optimal dietary concentration was 5 mg. per cent.

The effects of L-thyroxine and some of its analogues on lipid metabolism have been reviewed (*Nutrition Reviews* **17**, *303* (*1959*); **18**, *249* (*1960*)). W. R. Ruegamer and his group demonstrated that the analogue known as 3,5-diiodothyroacetic acid or "Diac" was capable of altering lipid metabolism in rats without significantly hampering weight gain or increasing oxygen consumption.

Recently, these same investigators reported an attempt to determine the optimal quantity of Diac which could be fed to rats in order to achieve normal concentrations of cholesterol in their blood and tissues without inducing hypermetabolism (W. R. Ruegamer and F. R. Silverman, *Endocrinology* **68**, *564* (*1961*)). The authors selected a purified diet containing 19 per cent protein as casein and 29 per cent fat as lard. To this high fat diet they added cholesterol at a level of 1 per cent. Weanling rats were fed this diet for three weeks and compared with rats fed Purina Chow. The experimental animals developed modest hypercholesterolemia but had extreme elevation of hepatic cholesterol and marked elevation of total lipid in the liver. When similarly fed animals were given Diac 500 μg. per 100 g. of body weight intraperitoneally or as a supplement of 5 mg. per 100 g. diet, it was found that the dietary route resulted in a greater reduction in concentrations of cholesterol and lipids.

Accordingly they designed a study to determine the optimal quantity of Diac to be added to a high fat diet containing cholesterol. They hoped to achieve normal concentrations of cholesterol in the blood and liver, yet avoid impairment of the rate of weight gain. Diac was fed to nine groups of weanling rats in concentrations varying from 0.4 mg. to 100 mg. per 100 g. diet. Measurements were made of the concentrations of cholesterol in the blood and liver and of the total lipids in the liver. Weight gain was determined weekly. It was found that the concentration of hepatic cholesterol was the most sensitive index of response. There was a sharp decrease in hepatic cholesterol when the 5 mg. per 100 g. level of Diac was reached. At the same time the total lipids in the liver decreased markedly and serum concentrations of cholesterol decreased modestly. Rate of weight gain was practically normal when this quantity of Diac was fed, but greater amounts of Diac resulted in progressively poorer rates of weight gain.

Next the authors studied the effects of Diac in animals already hypercholesterolemic as a result of previously eating the experimental diet. After three weeks of this diet the rats were given 5 mg. per 100 g. of Diac in their food and were noted to have sharp decreases in the concentration of cholesterol and lipid in the liver. Similar groups of rats were fed the atherogenic diet for three weeks after which time cholesterol was removed from their diet. They also showed substantial decreases in concentrations of cholesterol in their serum and liver. Another group of animals pre-fed the atherogenic diet for three weeks, had cholesterol removed from the diet and Diac 5 mg. per 100 g. added to the diet. This regimen resulted in extreme depression of the concen-

tration of cholesterol in the liver and substantial decreases in serum cholesterol and total hepatic lipids.

Next the authors studied the effects of prolonged feeding of Diac and the atherogenic diet. The few animals which died were found to have evidence of vitamin K deficiency. Since this vitamin had not been included in the diet and since it was noted that these animals had no significant degree of coprophagy, it was concluded that a vitamin K deficiency was the most likely cause of death. Inclusion of vitamin K in the diet corrected the defect and the experiment continued uneventfully. After five months the animals which were given 5 mg. per 100 g. of diet of Diac in the atherogenic diet had markedly lower concentrations of cholesterol in their serum and liver than the control animals. However, these concentrations still were considerably above normal. Their weight gain during the five month period was approximately normal. The aortas were taken from these animals and analyzed for cholesterol content, but no significant differences were noted. The authors concluded that Diac can reduce the amount of tissue deposition of cholesterol, at least in the liver of rats fed an atherogenic diet. They also concluded that Diac can reduce hepatic stores of cholesterol when given in doses which do not induce significant hypermetabolism.

The mechanism whereby thyroid hormones reduce cholesterol concentrations probably depends upon an increased rate of excretion (S. O. Byers, *Am. J. Clin. Nutrition* **6,** *642 (1958)*).

It will be of great interest to learn whether Diac can remove cholesterol deposits from the vascular tree of animals made atherosclerotic by prolonged feeding of an atherogenic diet.

LOWERING OF SERUM CHOLESTEROL BY AN ADIPHENINE DERIVATIVE

An adiphenine derivative, an inhibitor of cholesterol synthesis, reduced plasma total cholesterol in experimental dogs but caused fatty liver.

Current interest in hypercholesterolemia has stimulated the search for agents capable of reducing plasma cholesterol levels. Their discovery has been facilitated by the development of *in vitro* preparations for the biosynthesis of cholesterol. The finding that rat liver homogenates use the lactone of 2-C^{14}-mevalonic acid for cholesterol synthesis much more efficiently than did other precursors (P. A. Tavormina, M. H. Gibbs, and J. W. Huff, *J. Am. Chem. Soc.* **78,** *4498 (1956)*) immediately made possible the quantitative study of inhibitors of this system. As a result, a number of inhibitors have been found and shown to lower serum cholesterol levels, presumably because of reduced biosynthesis.

One of these agents is benzmalacene (Huff and J. L. Gilfillan, *Proc. Soc. Exp. Biol. Med.* **103,** *41 (1960)*). Another is triparanol; both have undesirable side effects. The present review concerns another, β-diethylaminoethyl diphenylpropylacetate hydrochloride ("SKF 525-A"), related to triparanol and studied by E. C. Dick *et al.* (*Proc. Soc. Exp. Biol. Med.* **104,** *523 (1960)*).

This drug is a derivative of the smooth muscle relaxant adiphenine ("trasentine") and has an interesting history (*Pharmaceutical Journal* **118,** *419 (1954)*). Early study showed that it had no spasmolytic activity and no apparent action of its own. However, it proved to prolong greatly the action of a variety of quite dissimilar drugs: barbiturates, the analgesics codeine and pethidine, and amphetamine. Further research indicated that the compound inhibits the metabolism of the drugs. In due course

"SKF 525-A" proved also to inhibit cholesterol synthesis. One later paper suggests that the metabolic block lies between isopentenyl pyrophosphate and squalene (W. L. Holmes and J. D. Bentz, *J. Biol. Chem.* **235,** *3118 (1960)*).

Dick *et al.* selected dogs with "naturally high" plasma total cholesterol values (above 150 mg. per 100 ml.) fed a commercial ration and whole milk. Five dogs received 20 mg. of the drug per kg. body weight per day for two and one-half weeks. In a second experiment three dogs received 10 mg. per kg. per day for 19 and one-half weeks. For each group there were five control dogs given placebos. Serum cholesterol and other lipids were measured, as well as a number of related parameters. Autopsies were performed.

In the dogs given the larger doses, the plasma total cholesterol level fell in two and one-half weeks from an initial value of 186 mg. per 100 ml. to about 30 mg. per 100 ml., a decrease of 84 per cent. The plasma phospholipid concentration followed this curve closely. The drug was stopped at two and one-half weeks and the plasma cholesterol returned to normal at the end of two more weeks.

The three dogs given the smaller doses (10 mg. per kg.) had a similar fall in plasma total cholesterol, with mean values initially, after three and one-half weeks' and after 19 and one-half weeks' treatment, respectively, of 216, 88, and 92 mg. per 100 ml. Other lipids were determined only at 19 and one-half weeks: plasma total lipids were then 770 mg. per 100 ml. (vs. 1,097 for the control dogs), phospholipids 183 (vs. 406 for the controls).

The serum alkaline phosphatase was increased in the treated animals at 17 weeks. Other tests of liver function were not significantly changed. The plasma protein-bound iodine level of the treated dogs was lower than that of the control animals.

Autopsies were described as normal except for the presence of fatty liver. The liver cholesterol content was increased, whereas the aortas contained a reduced amount. The adrenals are not specifically mentioned, though presumably sectioned.

Treatment was discontinued on one dog after 19 and one-half weeks. Plasma alkaline phosphatase returned to normal within three weeks, as did the plasma total cholesterol, which rose from 35 to 194 mg. per 100 ml. The animal was sacrificed at this time; its liver appeared normal on autopsy and had a normal total lipid content on analysis.

The authors point out that the evidence of liver damage provided by serum alkaline phosphatase measurements would have been missed had the generally less sensitive tests, bromsulfalein retention and serum bilirubin determination, been relied upon.

Dick *et al.* have also found that the drug lowers plasma cholesterol in rats, mice, and monkeys and produces fatty liver as well in the rats and mice (the monkey livers were not examined).

It appears clear that the drug inhibits cholesterol synthesis, lowers the plasma cholesterol level, produces fatty liver, alters thyroid function, and that these effects are all reversible in two or three weeks. The relation of cholesterol to the thyroid is not at all clear; here is one experimental method for producing hypocholesterolemia which may be worthwhile for future research.

The authors wisely make no claim for this drug in the management of human patients. However, it is well known that dietary fatty liver can lead in time to cirrhosis and there is no reason to doubt that the same result might follow from the use of SKF 525-A or other drugs which act similarly.

While the *in vitro* biosynthesis of cholesterol from mevalonate is a very useful test system for screening drugs, there are a dozen intermediate steps in this transformation and we do not know at which particular stage a given drug interferes with the process. Differences between drugs in this

regard may or may not prove to be physiologically important.

The clinical trial of drugs which interfere with cholesterol synthesis may be without danger over a period of a few weeks, but the possibilities of liver cirrhosis, interference with the synthesis of steroid hormones, and the quite unexplored effect on the thyroid, are important and potentially hazardous.

There is a real tendency for clinicians to infer that these drugs will prevent or alter the course of atherosclerosis. But it would be very difficult to substantiate this at present. Not only is cholesterol not the single determinant of atherosclerosis, but one would have to define and measure the pattern of various cholesterol precursors which accumulate when cholesterol synthesis is blocked, and then prove that each of these did not in itself contribute to atherosclerosis. In other words, the inhibitors of cholesterol synthesis are of great experimental interest, but their effects are too unexplored and potentially hazardous to justify their general use at present in patients with hypercholesterolemia.

RADIONUCLIDES, CALCIUM, AND POTASSIUM IN MILK

There is wide variation in the level of radionuclides and of calcium and potassium found in milk. Superimposed on the levels found is a marked seasonal variation.

The constant surveillance which the Public Health Service has been maintaining on the level of radionuclides occurring in milk from milksheds in various parts of the country, has yielded some interesting data on the variations which occur among these various milksheds. Further, the results have continued to show a rather striking seasonal effect upon the level of radionuclides in milk.

J. E. Campbell *et al.* (*J. Agr. Food Chem.* **9**, *117* (*1961*)) have reviewed the results obtained for the period between May 1957 and December 1959. In reviewing these data, Campbell *et al.* have attempted to draw some conclusions and to make some predictions with respect to the future levels which may be expected, and what these may mean in terms of the average human body burden.

Interestingly enough, the determination of strontium^{90} and cesium^{137} did not show any clear association with the publicly announced weapons tests. On the other hand, there was a very sharp correlation between weapons test activity and the occurrence in milk of radionuclides with short half lives. This correlation was observed in all areas and applied to iodine^{131}, barium^{140}, and strontium^{89}.

Within about three months after the last weapons test, all of the I^{131} and Ba^{140} had decayed to negligible values. However, the half life of Sr^{89} is long enough so that measurable concentration could be observed for about one year after the last test.

While it was not possible to show clear-cut seasonal variations during the weapons testing periods, following the cessation of weapons testing it was evident that there was a rather clear-cut seasonal variation in Sr^{89}, Sr^{90}, and Cs^{137}. Campbell *et al.* assume that these variations reflect the feeding patterns of dairy cattle and a shift from pasture to supplement feed. The peak was reached in April, and the lowest levels in late summer.

As a part of the program of analysis, calcium and potassium values have been determined for the milk samples from the various areas. There has been a significant difference in the concentration of calcium, both by months and by geographic location. The samples from the New York and Chicago areas were the lowest in calcium content, and those from St. Louis were the

highest. In no case did the calcium values fall below 1 g. per liter of milk, but in St. Louis during January, for instance, the calcium value was 1.33 g. per liter. The calcium values during the rest of the year were consistently higher for St. Louis than for the other sampling stations. On the other hand, the potassium values for milk samples from St. Louis were significantly lower than for samples from the other milksheds, with those from New York having the highest values. The authors make no attempt to evaluate the significance of these results, but it is obvious that the milk from the various milksheds of the United States is by no means a standard product when the mineral composition is the basis for comparison.

Campbell *et al.* have determined what the radionuclide values may mean in terms of the estimated intake of human beings and have compared this figure with the recommendations of the International Commission on Radiological Protection (*Health Physics* **3,** *1, (1960)*). If one takes the maximum permissible level of exposure for an average general population as proposed by this Commission, or 70 micromicrocuries of Sr^{90} per day, it is possible to point out that with the environmental Sr^{90} found in a given location in the United States, there is a level of exposure much lower than this figure.

The 70 micromicrocurie figure, however, can only be used as a point of reference since it does not take into account the availability of Sr^{90} in various food sources, the effect of other items in the diet, such as level of calcium, and such factors as variation in diet and age of the population. However, it is interesting to observe that the total daily Sr^{90} consumption under conditions existing in this country probably will not exceed 15.4 micromicrocuries.

The compilation and review of radionuclide values in milk made by Campbell *et al.* serve to emphasize that many factors may influence the level of radioactive isotopes present in the food of our population. The accumulated data indicate that the level of Sr^{90} in milk is decreasing, as is the level of Cs^{137}. It is probable that the maximum levels of human tissue accumulation, barring resumption of weapons testing, are likely to occur within the period 1960 to 1962.

NOTES

Blood Saccharoids

Techniques have been devised to form protein filtrates in which the nonglucose reducing material of blood is decreased. The evaluation of the glucose in the blood using copper reduction methods approached a true glucose level. The term saccharoids was used to cover a group of substances which appear in blood filtrates and reduce copper reagents but are not fermentable with yeast. They are nonglucose reducing compounds.

With the development of methods employing the enzyme glucose oxidase it is possible to determine specifically glucose in blood filtrates. Recently B. W. Volk, A. Saifer, and S. S. Lazarus (*J. Lab. Clin. Med.* **57,** *367 (1961)*) carried out a series of studies in which they determined the blood glucose levels in the same sample using the Nelson-Somogyi copper reduction method and the glucose oxidase procedure on barium-zinc filtrates. The difference, expressed in milligrams, between the glucose values obtained by these two methods, was called the blood saccharoid level. To determine the blood saccharoid levels, 80 blood samples obtained from 20 nondiabetic subjects were studied. The saccharoid level was found to be 6.0 ± 4.0 mg. per 100 ml. Ninety-eight per cent of

the saccharoid values determined were below 15 mg. per 100 ml. This was taken as the upper limits of normal. In a series of 35 diabetic patients in which 140 determinations were carried out the average saccharoid level was 2.10 ± 10.6 mg. per 100 ml. The range of the saccharoid values for the diabetic patient was 0 to 65 mg. per 100 ml. with 92 out of 140 samples falling above 15 mg. per 100 ml. In any individual patient the level of the saccharoid was the same whether determined in the capillary or in the venous blood.

Studies were carried out attempting to modify the blood glucose level, either by doing a modified glucose-insulin tolerance test or by administering tolbutamid. These tests were carried out on 12 diabetic and six nondiabetic individuals. In the modified glucose-insulin tolerance test the blood sugar rose and then fell, but the saccharoid level remained relatively constant. Thus, neither glucose nor insulin influenced the level of blood saccharoids. Also, the level of the saccharoids in the blood was found to be independent of the patient's sensitivity to insulin. The administration of 1 g. of tolbutamide orally causes a fall in the blood sugar. However, it was found that the fall in the copper reducing method paralleled the fall noted in the blood glucose levels when glucose oxidase was used. Thus, the saccharoid level appeared to be independent of the extent to which the blood sugar was lowered.

These investigators then made a study of the chemical nature of the blood saccharoid material. It was possible to concentrate the unknown material by lyophilizing a large quantity of protein-free filtrate of venous blood obtained from two patients, one with a saccharoid level of about 30 mg. per 100 ml. and the other with a level of about ten. Then they carried out a series of chromatographic analyses. No spot of sugar other than glucose was noted on the chromatograms. However, in testing another aliquot for organic acids, they detected a material which had the same Rf value as gluconic and glucuronic acids. They also noted that larger amounts of this material appeared in the filtrates from diabetic than from nondiabetic samples. It should be noted that no blood from either group contained pyruvic acid.

No further attempt was made to characterize the saccharoid material. However, older data in the literature indicate that the nonglucose material of the blood which reduces copper contains glucuronic acid. This organic acid has a copper reducing equivalent which is practically identical with that of glucose. Glucuronic acid is neither precipitated by heavy metal salts nor fermented by yeast. Hence, these investigators believe that the blood saccharoids may be glucuronic acid. If future studies indicate the mechanism by which the saccharoid level is increased in the diabetic patient, this test may perhaps have some value in evaluating diabetes.

Environmental Temperatures and Urinary Metabolites of Noradrenaline

In newborn mammals R. E. Moore and M. C. Underwood (*Lancet* **1,** *1277* (*1960*)) reported that noradrenaline is thermogenic and may play a role in normal temperature regulation, compensating for changes in the environmental temperature. M. Sandler, C. R. J. Ruthven, I. C. S. Normand, and R. E. Moore (*Lancet* **1,** *485* (*1961*)) investigated the occurrence of this mechanism in the regulation of body temperature of the newborn infant.

Since they did not consider it practical to administer noradrenaline to the newborn, these investigators used an indirect approach. They measured the effect of changes in environmental temperature on the urinary excretion of a metabolite of both adrenaline and noradrenaline. The metabolite chosen was 3-methoxy-4-hydroxymandelic acid, because it could be determined accurately and because a greater proportion of the pressor

amines appears in the urine in this form than in the free form.

They studied 16 normal full term male infants between the ages of four and nine days. The infants were placed in one of two thermostatically controlled incubators, one maintained at 85°F and the other at 75°F. Each infant spent about 21 hours per day in the incubator, with about three hours outside during which time he was fed, bathed and diapered. Nine of the infants spent the first day in the incubator at the higher temperature followed by a day in the incubator at the lower temperature. The seven remaining infants were subjected to the temperature changes in the reverse order, the first day at the lower temperature followed by the next day at the higher one. Complete urine collection was obtained from each infant during the study and the amount of 3-methoxy-4-hydroxymandelic acid excreted was determined.

By subtracting the amount of 3-methoxy-4-hydroxymandelic acid excreted while the infant was maintained at 85° from that excreted during the period at 75° a mean difference in excretion dependent on temperature was obtained. It was found that the difference was 0.087 ± 0.025 mg. per day (mean ± standard deviation). This was an increase of 25 per cent over the amount excreted at the higher temperature and the difference was statistically significant ($P < 0.01$). The average urine volume at the lower temperature was also greater than that noted at the higher temperature (59.3 ± 6.1 ml. per day). This difference was highly significant ($P < 0.001$). However, if the amount of 3-methoxy-4-hydroxymandelic acid excreted in the urine was expressed as milligrams per liter of urine, there would be no difference in excretion rate dependent on the environmental temperature.

Evidence is available that the amount of 3-methoxy-4-hydroxymandelic acid excreted in the urine of the adult is related to the output of catecholamines. Whether this assumption is valid in the newborn is not known. Also, this acid can be derived from both adrenaline and noradrenaline but it is not possible to determine whether the increased amount which occurs in the urine of infants maintained at the lower temperature comes from one or both of these pressor amines. However, these authors believe that the extra amount of 3-methoxy-4-hydroxymandelic acid found in the urine under these conditions is related to an increased excretion of noradrenaline.

Recent Books

Cancer Research Program of the Division of Biology and Medicine. U. S. Atomic Energy Commission. Available from Office of Technical Services, Department of Commerce, Washington 25, D. C. Pp. 94. Price $2.25.

The Family. A Focal Point in Health Education. The New York Academy of Medicine. Editor, Iago Galdston, M.D. Published by International Universities Press, Inc., New York City. Pp. 216. Price $3.00.

Foetal and Neonatal Physiology. The subject of a special issue of the British Medical Bulletin, May 1961 (Vol. 17, No. 2), is foetal and neonatal physiology. Published by the Medical Department, The British Council, 65 Davies Street, London, W.1. Pp. 98. Price $3.25.

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